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Effect of cladribine treatment on β-2 microglobulin and soluble intercellular adhesion molecule 1 (ICAM-1) in patients with multiple sclerosis

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β-2 Microglobulin (β2M) is a low molecular weight protein located extracellularly and associated with class 1 antigens of the major histocompatibility complex and is considered a marker for disease activity in immune disorders. Cladribine (2-chloro-2-deoxyadenosine, 2-CDA) is a potent lymphocytotoxic agent under investigation in the treatment in MS patients. As β2M levels may indicate inflammatory events in CNS we determined CSF-β2M and serum β2M levels in patients with relapsing-remitting MS before and after cladribine treatment as well as in a control group. There was a significant β2M decrease in sera but not in CSF in MS patients after the cladribine treatment, associated with a slight but significant clinical improvement measured by Kurtzke's Expanded Disability Status Scale. We also found a significant decrease in sICAM-1 level in CSF but not in sera in MS patients. The data support a role of cladribine in MS therapy and deliver new information on cladribine immunological effects in MS patients.

key words: multiple sclerosis, β-2 microglobuline, cladribine, ICAM-1

INTRODUCTION

Multiple sclerosis (MS) is one of the most common demyelinating disorders of the central nervous system (CNS). Its pathomechanism remains still unknown. It is suspected to be of autoimmune nature and many observations indicate involvement of T lymphocytes [6, 9]. There is evidence that in the first stages of the disease lymphocytes migrate from the vascular bed across the damaged blood-brain barrier to the brain tissue and cause myelin destruction [6, 9, 11]. The inflammatory response and consequent demyelinating lesions lie in close relationship with cerebral vessels, particularly the veins and venules [20]. Moreover it has been confirmed that perivascular inflammation is more widespread than demyelinating plaques themselves, which suggests altered functioning of cerebral endothelium in early MS [20].

Leukocyte-endothelial interactions are regulated by several adhesion molecules, including selectins, integrins and molecules of the immunoglobulin superfamily. Some of them, e.g. vascular cell adhesion molecule (VCAM-1), appear on the surface of endothelial cells only after activation by cytokines (interleukin-1, interferon gamma, tumour necrosis factor), some are present on resting endothelium, like

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intercellular adhesion molecule (ICAM-1), but their expression increases during inflammation. ICAM-1 and other members of the immunoglobulin superfamily are involved in strong leukocyte binding to the endothelial cells and allow their transmigration. Many investigators have found increased concentrations of the soluble form of ICAM-1 in patients suffering from MS [8, 9, 11] and correlated it with disease activity. This suggests that the expression of this molecule is strictly implicated in the pathomechanism of the disorder. Moreover, soluble forms of these cell adhesion molecules are probably involved in the negative feedback, reducing leukocyte-endothelium adhesion and consequently their migration across the vascular wall toward the inflamed brain tissue.

β-2 microglobulin (β2M) is a low molecular weight protein located extracellularly and associated with class 1 antigens of the major histocompatibility complex. There are reports that strongly suggest its role in the activation of the immune system [17]. β2M is then considered a marker for disease activity in immune disorders [1]. Its precise role in pathology remains still unknown, but it is supposed that it may be implicated in lymphocyte activation in MS. Previous studies showed β-2 microglobulin to be increased in cerebrospinal fluid (CSF) and sera of these patients [2, 5].

Cladribine (2-chloro-2-deoxyadenosine, 2-CDA) is a potent lymphocytotoxic agent under investigation in the treatment of MS patients, earlier successfully used in hairy-cell-leukemia and other autoimmune disorders therapy [3, 7, 15]. There is evidence that it could be effective in reducing activity in both chronic progressive MS and the relapsing-remitting form of the disease [10, 18, 19]. The purpose of our paper is to study the effect of cladribine treatment on β-2 microglobulin and soluble intercellular adhesion molecule 1 (ICAM-1) in patients with multiple sclerosis to increase the knowledge of immunological consequences during this therapy.

MATERIAL AND METHODS

We studied 25 patients (age range 20 to 50 years, 17 females and 8 males) diagnosed as definite MS according to Poser's criteria [20]. All had the relapsing-remitting form of the disease. They had 2.5 ± 0.5 relapses within the last 2 years with an average disease duration of 4.5 ± 2.2 years.

The patients were given 5 mg 2-CDA in one daily subcutaneous injection for 5 days each in 6 monthly courses.

None of them had been treated with immunosuppressive therapy 6 months before inclusion. A new relapse was the cause of admission of all patients. Paired CSF and serum samples were drawn from all MS patients before therapy (during a relapse) and on the day of the last injection of 2-CDA (during another relapse in 4 patients and out of relapse in 21 of them). The disability status of MS patients was determined by Kurtzke's expanded disability status scale EDSS [12]

The control group consisted of 10 sex- and age-matched patients (age range 20 to 50 years, 7 females and 3 males) from whom CSF was drawn to exclude neurological disorders and who were then diagnosed as tension type headache.

Both CSF and blood samples from MS patients and controls were centrifuged at 1200 rev/min for 10 min and stored at –70°C. The concentration of sICAM-1 in the CSF and serum was measured by ELISA method (sICAM-1 Quantitative Colorimetric Sandwich ELISA, R&D Systems, USA). β-2 concentration in the CSF and serum was determined by β-2 microglobulin sandwich ELISA method [13]. IgG CSF levels and IgG indices were determined as well.

Statistical analysis was carried out using Wilcoxon test for matched pairs; p-values lower than 0.05 were considered significant; the Mann-Whitney U-test was used for the comparison between the MS group before treatment and controls.

RESULTS

The mean value of the EDSS score in MS patients before treatment was 3.5 ± 2.4. It decreased after the therapy to a value of 2.7 ± 2.1 and reached the limit of statistically significant difference (Table 1). There was also a statistically significant decrease in CSF IgG concentration as well as in IgG index after the therapy with 2--CDA (Table 2). We observed a significant reduction of β2M serum level and a decrease in the CSF sICAM-1 concentration after cladribine treatment (Table 3).

Table 1. Clinical status of MS patients (EDSS) before (a) and after (b) treatment

All MS patients (25)	EDSS (a)		EDSS (b)
	3.5 ± 2.4	⟺	2.7 ± 2.1 p = 0.048
No relapse after treatment (n=21)	3.6 ± 2.3	⟺	2.6 ± 1.8 p = 0.03
Relapse after treatment (n = 4)	3.1 ± 2.5		3.2 ± 1.6

Table 2. CSF IgG concentration and IgG index before (a) and after (b) treatment in MS patients and control group

	IgGa (mg%) Mean ± SD	IgGb (mg%) Mean ± SD	IgG index (a) Mean ± SD	IgG index (b) Mean ± SD
All MS patients (n = 25)	5.17 ± 3.67	4.81 ± 2.35	1.11 ± 0.75	1.01 ± 0.59
Control group (n = 10)	2.5 ± 0.9 p = 0.01		0.5 ± 0.09 p = 0.01	

DISCUSSION

We found for the first time that after 2-CDA treatment β2M level is significantly decreased in serum of MS patients. However there was no difference between MS patients and controls with respect to serum and CSF β2M concentration.

These observations are in accordance with those of Ott and colleagues [14] and in contrast with some previous reports [4]. We conclude that β2M may be useful in evaluation of 2-CDA treatment in MS patients and cannot be considered a specific diagnostic marker for MS.

Since we did not find any elevation of β2M CSF/serum ratio (it ranged from 0.38 to 0.48 and was slightly lower than that reported by Adachi [1]) and noting that β2M is a very low molecular weight protein which easily passes through the blood-brain barrier, it is likely that β2M presence in CSF is due to its transudating from blood rather than to its intrathecal synthesis. This is consistent with the finding that a significant decrease in β2M level was found only in serum but not in CSF.

An inverse observation was made when measuring sICAM-1 levels. In contrast to some previous reports [8, 9,11] we did not find a significant difference between sICAM-1 concentration in MS patients compared with controls. Nevertheless, we found for the first time a significant decrease in CSF sICAM-1 level after 2CDA treatment only in the group of patients without relapse after the therapy, but not in sera in either group of patients (with or without relapse at the end of the treatment). Noting that sICAM-1 reflects the expression of its cell-bound form (ICAM-1), which is involved in leukocyte trafficking across the blood-brain barrier, it seems that a significant reduction of sICAM-1 may be a result of the downregulated expression of ICAM-1.

Table 3. β-2 microglobline (β2M) and sICAM–1 before (a) and after (b) treatment in MS patients

	β2M mg/l (a) Mean (range)		β2M mg/l (b) Mean (range)		sICAM–1 ng/ml (a) Mean (range)		sICAM–1 ng/ml (b) Mean (range)	
MS (25)	0.76		0.60		4.33		1.65	
CSF	(0–2.65)	SD= 0.67	(0–3.03)	SD= 0.69	(0–18.5)	SD = 4.2	(0–8.4)	SD = 2.0 p = 0.0036
Without relapse after treatment (n = 21)	0.74 (0–2.48)	SD = 0.61	0.61 (0–2.64)	SD = 0.58	4.42 (0–17.2)	SD = 3.6	1.60 (0–8.1)	SD = 1.8
Relapse after treatment (n = 4)	0.77 (0–2.65)	SD = 0.69	0.59 (0–3.03)	SD = 0.62	4.30 (0–18.5)	SD = 4.4	4.1 (0–8.4)	SD = 4.3
MS (25)	1.7		1.54		412.8		371.8	
Serum	(0.88–2.92)	SD = 0.54	(0–4.49) p = 0.046	SD = 0.95	(230.5–598.3)	SD = 98.6	(49.5–761.1)	SD = 154.9
Without relapse after treatment (n = 21)	1.68 (0.88–2.85)	SD = 0.50	1.39 (0.5–3.94) p = 0.038	SD = 0.82	413.1 (230.5–597.4)	SD = 96.8	389.2 (49.5–735.3)	SD = 131.0
Relapse after treatment (n = 4)	1.72 (0.75–2.92)	SD = 0.60	1.70 (0–4.49)	SD = 2.62	408.6 (241.2–598.3)	SD = 412.8	400.8 (67.4–761.1)	SD = 406.4
Controls (10)	0.8				4.61			
CSF	(0.1–1.58)	SD = 0.6			(1.59–17.8)	SD = 4.74		
Controls (10)	1.61				432			
Serum	(0.1–3.1)	SD = 0.96			(201–863)	SD = 222		

The EDSS improvement that we observed during the study is in accordance with other investigators' results [18, 19]. We found a similar reduction of the EDSS score in our MS patients, as did Grieb and colleagues [10]. The findings support a role of cladribine in MS therapy and deliver new information about cladribine immunological effects in MS patients, but further studies on that subject are necessary.

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